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The Effects of Relaxation Techniques on Salivary Measures in Student Pharmacists

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Abstract

- In 2011, the Accreditation Council for Pharmacy Education[1] mandated measurement of stress in Student Pharmacists, specifically potential negative impacts on learning experiences and morale.

- Votta and Benau (2013)[2] provided supporting evidence that pharmacy students experience elevated, but not extreme, levels of perceived stress as compared to the general population.
- Leffler *et al.* (2016)[3] imposed a relaxation or non-relaxation technique on student pharmacists - they found two relaxation techniques... **Read More**

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Background

- In 2011, the Accreditation Council for Pharmacy Education[1] mandated measurement of stress in Student Pharmacists, specifically potential negative impacts on learning experiences and morale.
- Votta and Benau (2013)[2] provided supporting evidence that pharmacy students experience elevated, but not extreme, levels of perceived stress as compared to the general population.
- Leffler *et al.* (2016)[3] imposed a relaxation or non-relaxation technique on student pharmacists – they found two relaxation techniques (Mindfulness and Body Scan Meditation) and one control (Power Posing) were rated as easiest to conduct, most liked, and to cause subjects to feel more relaxed. In contrast, Mental Stimulation and 4 x 4 breathing were found to be the least useful to reduce stress.
- Salivary measures were determined in these same student pharmacists, to evaluate whether they provide similar outcomes when evaluating the effects of relaxation and non-relaxation techniques on student pharmacists as compared to survey results.

Objectives

- **Primary outcomes**: To investigate percent changes in physiological measures of stress (salivary cortisol and alpha-amylase) and power (salivary testosterone) when student pharmacists conduct relaxation and non-relaxation techniques.
- **Secondary outcomes**: To determine whether changes in physiological measures correlate to changes in perceptions of stress, anxiety, and technique evaluation as determined by survey.

Methods

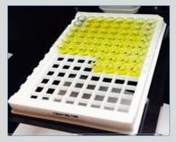
- **Population**: **86 Student Pharmacists** (51 females and 35 males) were recruited from P1 (n=29), P2 (n=26), and P3 (n=31) years from the SWOSU College of Pharmacy in Spring 2016.
- **Techniques assigned to evaluate primary and secondary outcomes:**
  - Three relaxation interventions (n = 48)
    - **Body Scan Meditation(n = 16)** – Verbally-guided systematic muscle relaxation.
    - **Mindfulness Meditation (n = 16)** – Focusing on the sound of a bell.
    - **4 x 4 Meditation (n = 16)** – Breathing slowly in and out for four counts.
  - Two non-relaxation controls (n = 38):
    - **Power Posing (n = 19)** – Physically holding an open pose[4].
    - **Mental Stimulation (n = 19)** – Playing Word Streak App (www.zynga.com/games).
- **Protocol**:

Pre
- Survey perceived stress, anxiety, & technique opinions.
- Collect saliva sample.

Treat
- Conduct Relaxation or Non-Relaxation Technique (8 to 10 min).
- Record physiological measures (HR, RR, tension, temperature).

www.salimetrics.com

Post
- Survey perceived stress, anxiety, & technique opinions.
- Collect saliva sample.
- Aliquot each salivary sample into two cryovials and freeze at -20°C until analyzed for physiological components.



- **Lab Analysis**: Salivary samples were analyzed using commercial kits (Salimetrics, LLC) for Cortisol (an increase suggests an endocrine stress response[4]), alpha-Amylase (surrogate measure for Sympathetic Nervous System[5]), and Testosterone (an increase suggests increased feelings of power[4]), and) via a Synergy H1 Hybrid microplate reader (BioTek Instruments, Inc.). Intra-assay and inter-assay coefficient of variations for cortisol (5.32% and 7.30%) and testosterone (5.23% and 11.94%), respectively, were within acceptable limits as recommended by Salimetrics (LLC).
- **Statistical Analysis:** All data were analyzed using IBM SPSS Advanced Statistics Software, version 23.
  - SPSS GLM univariate analysis was used to evaluate primary outcomes, with technique, gender, year, and previous meditation experience investigated as independent variables.
  - Secondary outcomes were evaluated utilizing Spearman's correlation analysis.
  - Based on survey results, it is hypothesized Power Posing, Mindfulness and Body Scan Meditation will physiologically result in lowered stress levels (via declines in cortisol and alpha-amylase), whereas Power Posing, 4 x 4 breathing, and Mental Stimulation will result in higher power levels (via testosterone). Positive correlations are expected between cortisol and alpha amylase with stress and anxiety, but negative correlations with other survey items. Testosterone will be opposite.

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Results

- **Primary outcomes**: Physiological comparisons of relaxation versus non-relaxation techniques were not different (t test; $P > 0.12$). Therefore, salivary measures were compared across techniques.

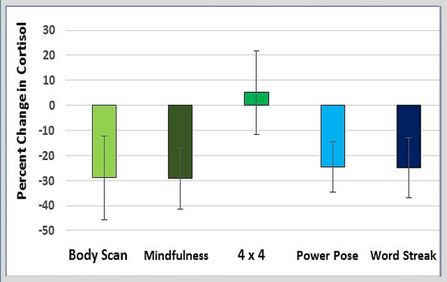

Figure 1. Means and SE of percent change in salivary cortisol (Technique, $P = 0.27$).

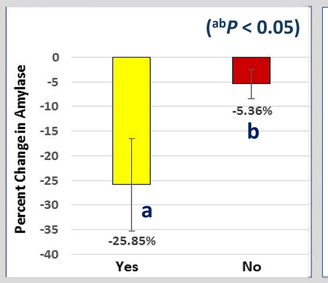

Figure 2. Means and SE of percent change in salivary amylase [Previous meditation experience (n=9) versus no experience (n=74), $P = 0.04$].

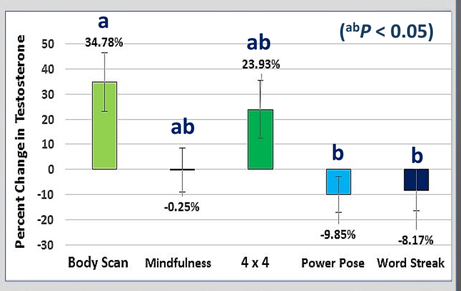

Figure 3. Means and SE of percent change in salivary testosterone (Technique, $P = 0.006$).

- **Secondary outcomes**:

Table 1. Spearman correlation coefficients for percent change in physiological measures as related to each other and to change in survey ratings.

Variable	% Change Cortisol	% Change Amylase	% Change Testosterone
Change in Salivary Cortisol	--	0.108	**0.434****
Change in Salivary Amylase	0.108	--	0.033
Change in Salivary Testosterone	**0.434****	0.033	--
Change in Perceived Stress Level[a]	**0.232***	0.048	0.163
Change in Perceived Anxiety Level[a]	0.058	-0.061	-0.019
Change in Feeling More Relaxed[a]	-0.038	-0.104	-0.042
Change in Perceived Easiness to Conduct the Technique[a]	-0.094	-0.027	0.013
Change in Perceived Usefulness of Technique to Reduce Stress[a]	-0.093	0.020	0.021
Change in Perceived Likeability of Conducting the Technique[a]	-0.077	-0.046	0.000
Change in Likelihood to Use the Technique in the Future[a]	0.015	-0.052	0.035
Change in Likelihood to Recommend the Technique to Others[a]	0.001	-0.040	**0.205**
Change in Likelihood to Recommend Relaxation to Others[a]	0.045	-0.062	-0.107

[a]Survey items rated by subjects as to level of agreement (Likert Scale, 1 to 5) before and after conducting their assigned technique. Higher ratings indicate a higher self-reported stress or anxiety level, or relate to a higher level of agreement to the survey item.
**Correlation is significant at the 0.01 level (2-tailed).
*Correlation is significant at the 0.05 level (2-tailed).

Conclusions

- **Salivary measures of stress (alpha-amylase and cortisol) were decreased across techniques.**
- **Physiological measure of power (testosterone) was increased in the more physically-active relaxation techniques, but was not increased in the non-relaxation techniques.**
- **Change in cortisol was significantly, positively correlated with testosterone and stress level only.**
- **Large variations in salivary measures made it difficult to detect significant changes. This has been observed in other studies we have conducted[6,7].**
- **Future analysis will integrate other physiological measures (HR, RR, temperature, and muscle tension) and results from other survey instruments (Kentucky Inventory of Mindfulness Skills, Four Scale Anxiety Questionnaire, and Perceived Stress Scale).**
- **Findings will enable us to plan future studies and ultimately make evidence-based**

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